Respiration and oxidation of various substrates by ileum in vitro[1,2]

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NEPTUNE, EDGAR M., JR. Respiration and oxidation of various substrates by ileum in vitro. Am. J. Physiol. 209(2): 329-332. 1965. The respiration of ileum from the rabbit, monkey, hamster, guinea pig, and rat in vitro was studied. Measurements were also made of the rate of oxidation of labeled glucose, glutamine, acetate, and palmitate to $C^{14}O_2$ by ileum in these species. Added glutamine and acetate are oxidized at a faster rate than added glucose by rabbit ileum. The guinea pig and monkey ileum gave values similar to the rabbit except that the guinea pig tissue had a higher rate of respiration. The hamster and rat ileum both oxidized glutamine and glucose at nearly the same rate. All species oxidized acetate vigorously. All species oxidized labeled palmitate which was added at low concentrations. Mucosal cells of rabbit ileum oxidized the various substrates at rates that were proportional to full-thickness ileum. It is concluded that ileum from various species of animals can oxidize a number of different substrates in vitro. There are differences in rates of oxidation by various species.

oxidation of glucose   glutamine   glutamate
palmitate   acetate   intestinal metabolism   ileum

In a previous communication from this laboratory, evidence was presented that glucose did not stimulate sodium transport in the everted sac prepared from rabbit ileum; glutamine, however, did stimulate sodium transport (4). This raised the possibility that glutamine might be a better substrate than glucose for oxidative metabolism of rabbit ileum in vitro. Therefore, some observations were made of the oxidative and respiratory characteristics of rabbit ileum and ileum from other species and these are reported in this communication.

EXPERIMENTAL METHODS

All animals were fed ad libitum, except where noted. Rabbit ileum was obtained from local rabbits that were

anesthetized with Nembutal. A segment of ileum was quickly excised and placed in an oxygenated buffered salt solution at room temperature that was identical to the solution used in the experimental period except that no substrate was added. The segment was opened by a longitudinal incision and placed on a board with the mucosal side up. Discs of ileum were obtained by using a cork borer that cut out pieces weighing approximately 100 mg. Fresh wet weight was determined gravimetrically, and the tissue was immediately transferred to the incubation flask. Ileal segments from the monkey were obtained in the same way as used for the rabbit. Guinea pigs, rats, and hamsters were anesthetized with ether and segments were cut with scissors. Mucosal cells were prepared by pushing a microscope slide down the serosal surface of a segment of ileum and expressing the cells from the cut end of ileum, much as one would squeeze a tube of toothpaste. Microscopic examination of the intestine revealed that only apical cells had been removed.

Conventional Warburg flasks with triple side arms were used except for the determination of the respiratory quotient; in the latter determination, Dixon-Keilin flasks were used (5). The gas phase was oxygen. After introduction into the 37 C metabolic bath, 20 min was allowed for thermal equilibration; then the substrate was tipped in from a side arm. At the end of the incubation, the reaction was stopped by tipping in 0.1 ml 50% phosphoric acid. The flasks were allowed to shake an additional 15 min to ensure complete trapping of the CO_2 by the alkali in the center well. All oxygen consumption values are expressed as microliters per 100 mg wet wt per hr except for the apical cell preparation. In the latter, the cell dispersions were uneven and therefore rates of oxygen consumption were not comparable, so the radioactivity in the CO_2 is expressed as counts per minute per micromole oxygen consumed.

Incubation medium was composed of sodium phosphates 37 mM, NaCl 70 mM, KCl 5 mM, and $MgCl_2$ 1 mM. Glutamine, glucose, and acetate were added at a concentration of 74 mM. Palmitate was 0.037 mM and was added as the albumin complex (6). All acidic components were converted to the sodium salts, except for palmitate which was the potassium salt. The total volume

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TABLE I

Added Substrate	Oxygen Consumption, µl 100 mg per hr		C14O2, Counts min per 100 mg per hr	
	A	B	A	B
None	45	100		
Glucose-C14	69	110	2,400	3,900
Glutamine-C14	106	130	33,500	33,000
Glucose-C14 + Glutamine	81	Not done	420	Not done
Glutamine-C14 + Glucose	113	Not done	39,000	Not done
Palmitate-C14	Not done	110	Not done	126,000
Acetate-C14	Not done	105	Not done	39,000

A: respiration of rabbit ileum and conversion of glucose-C14 and glutamine-C14 to C14O2 (performed in January 1964). B: respiration of rabbit ileum and conversion of various substrates to C14O2 (performed in May 1964). The incubation medium was composed of sodium phosphate 37 mM, NaCl 70 mM, KCl 5 mM, and MgCl2 1 mM. Glutamine, glucose, and acetate were added to a concentration of 74 mM. Palmitate was 0.037 mM and was added as the albumin complex. The total volume of the incubation mixture was 2.7 ml; the pH was 8.0; the gas phase was oxygen. Duplicate flasks yielded values with no more than a 5% variation. Complete replicate experiments yielded values with no more than a 12% variation when done at the same time of the year. Despite this variation, the proportional response to the various added substrates was the same. All values are expressed as microliters or counts per minute per 100 mg fresh wet wt/hr.

TABLE 2. *Oxidation of C14-labeled substrates by apical luminal cells of rabbit ileum*

Added Substrate	Counts, min in CO2 per µmole O2
Glucose-C14	172
Glutamine-C14	1,240
Palmitate-C14	5,300
Acetate-C14	1,400

Apical mucosal cells were suspended in a buffered salt solution as described in Table 1. The substrate concentrations were as noted in Table 1. The cell dispersions were uneven and therefore oxygen consumption varied considerably, but when the data were expressed as counts per minute in CO2/µmole O2 consumed, there was excellent agreement between replicate flasks.

of the incubation mixture was 2.7 ml and the pH was 8.0.

Although the medium used here is hypotonic, several key experiments were repeated with an isotonic medium (NaCl = 140 mM) and essentially identical results were obtained.

The radioactivity in the CO2 was determined by precipitation as BaCO3 and counting as an infinitely thick sample in a proportional gas-flow counter (5).

Tracer amounts of labeled compounds were added at about 1 µc/flask. The initial activity of each substrate was measured and the radioactivity in the CO2 was adjusted on the basis of an initial 250,000 counts per flask.

Substrates were obtained from commercial sources. Only the palmitic acid-1-C14 was checked for purity. On thin-layer chromatography this material had a R_F iden-

tical to palmitic and oleic acids and all of the applied radioactivity was recovered from the spot when eluted with methanol with the use of a technique described elsewhere (3). This would indicate that the material described in the rest of this paper as "palmitate" is at least a mixture of labeled long-chain fatty acids, if not pure palmitate. Unfortunately, gas liquid chromatography that will resolve methyl palmitate from closely related methyl esters is not currently available to our laboratory. The glucose and L-glutamine were randomly labeled. The DL-glutamate was labeled in the 3,4 position. Sodium acetate was labeled on the carboxyl carbon.

All flasks were prepared in duplicate. Each experiment was repeated three times except for monkey ileum which was performed twice. In the hamster and guinea pig in one experiment lower rates of oxygen consumption than shown in Table 4 were noted; these lower rates are tentatively attributed to inadvertent anoxia during preparation of the tissue. The data portrayed in the tables represent values from one typical experiment which are validated by the replicate experiments.

RESULTS

The data in Table 1, A, on rabbit ileum compare the oxidation of glucose-C14 and glutamine-C14. Although glucose is oxidized to C14O2, glutamine is oxidized to C14O2 to a far greater extent. Glucose does not depress the oxidation of labeled glutamine but glutamine does inhibit the conversion of labeled glucose to C14O2 when both substrates are added together.

As shown in Table 1, B, rabbit ileum is also capable of oxidizing palmitate and acetate to CO2. Although the labeled palmitate was added in essentially tracer concentration, the acetate was added at a concentration that was equimolar with glutamine. Since the radioactivity in the CO2 is nearly identical for both acetate and glutamine, acetate is as vigorously oxidized as glutamine. Similar data are included in Table 2 in which apical luminal cells were studied. It is apparent that the apical cell has oxidative characteristics very similar to the segments of ileum.

In Table 3, 4 and B, are data which indicate that unlabeled glucose does not depress the oxidation of labeled acetate or labeled palmitate. Unlabeled glutamine does depress the oxidation of both acetate and palmitate.

As shown in Table 3, C, glutamate stimulates respiration of rabbit ileum but its conversion to C14O2 is only 10% of the conversion of glutamine to C14O2. Unlabeled glutamine depresses the conversion of glutamate to CO2, whereas the addition of glutamate does not markedly depress the oxidation of glutamine. Even if the glutamate values are increased twofold on the assumption that only the L isomer is oxidized, the general observations are still valid.

Since the rabbit ileum had a very appreciable respiration when no substrates were added, the respiratory quotient was estimated several times using full-thickness segments of ileum without added substrate. On all oc-

casions the RQ values were unity or greater (1.0 1.4) for normal rabbits. Single experiments (in duplicate) gave values of 0.85 for monkey, 0.85 for rat, 0.70 for guinea pig, and 0.87 for hamster.

In Table 4 are values for the oxidation of various substrates by ileum from the rat, guinea pig, hamster, and monkey. The oxygen consumption for the rat and monkey are in the same range as that for the rabbit, whereas the oxygen consumption of guinea pig and hamster is much greater. On several occasions lower rates of oxygen consumption were observed for the latter two species; this may have been caused by unintentional periods of anoxia during preparation of the tissue.

Glucose is oxidized by ileum of all four species. When the radioactivity in the CO_2 is calculated as counts per unit of oxygen consumed, there is very little species difference.

Glutamine is oxidized to CO_2. On the basis of units of oxygen consumed, the monkey and guinea pig oxidize glutamine at twice the rate of that for the hamster and rat. The rabbit ileum, however, oxidizes glutamine at a faster rate than all species tested. All four species oxidize

TABLE 3

Added Substrate	Oxygen Consumption, μl, 100 mg per hr	$C^{14}O_2$, Counts/min per 100 mg per hr
A: Oxidation of acetate-1-C^{14} by rabbit ileum		
None	75	
Acetate-C^{14}	75	4,000
Acetate-C^{14} + Glucose	85	4,600
Acetate-C^{14} + Glutamine	90	2,800
B: Respiration and oxidation of palmitate-C^{14} by rabbit ileum		
None	80	
Palmitate-C^{14}	78	50,000
Palmitate-C^{14} + Glucose	98	54,000
Palmitate-C^{14} + Glutamine	107	15,500
C: Respiration and oxidation of glutamate-C^{14} and glutamine-C^{14} by rabbit ileum		
None	74	
DL-Glutamate-C^{14}	91	2,300
L-Glutamine-C^{14}	95	22,400
DL-Glutamate-C^{14} + L Glutamine	101	760
L-Glutamine-C^{14} + DL-Glutamate	99	18,800

All experiments conducted in March 1964. For details see Table 1.

palmitate; the highest rate was found with hamster ileum. Although palmitate was added at trace concentrations, acetate was added at the same concentration as glucose, and acetate is vigorously oxidized to CO_2.

In the in vitro preparations, the oxidation of added substrates is in the following order:

monkey:	acetate > glutamine > glucose
hamster:	acetate > glutamine = glucose
guinea pig:	acetate > glutamine > glucose
rat:	acetate > glutamine = glucose
rabbit:	acetate = glutamine > glucose

DISCUSSION

From these studies of the ileum in vitro it is evident that ileal tissue is capable of oxidizing a variety of substrates. Glutamine is vigorously oxidized by the rabbit ileum as well as enhancing sodium and water transport in vitro (4). The mechanism for the preferential combustion of glutamine compared to glucose has not yet been elucidated, but it is reasonable to postulate that by deamidation and transamination glutamine is converted intracellularly to α-ketoglutarate and enters the citric acid cycle. The less marked oxidation of glutamate may well be attributed to the dicarboxylic moiety and reduced cellular permeability due to the terminal charges at each end of the molecule. Confirmation of this speculation must await experiments with cell-free preparations.

Although it is tempting to suggest that in vitro glucose is a substrate of minor importance, it must be kept in mind that ileum from all of the species studied has a very appreciable oxygen consumption in the absence of added substrates; furthermore, rabbit ileum has an RQ of unity or greater in the absence of added substrates. Therefore, if there is endogenous carbohydrate intracellularly in the ileum, there may well be a very significant intracellular dilution pool. Thus, the values reported here may be only true minimal values for glucose. Also, the present studies did not include any measurement of lactate formation. Dickens and Weil-Malherbe (1) reported very high rates of glycolysis both aerobically and anaerobically for rat ileum in vitro with and without added glucose.

Therefore there may well have been considerable energy for metabolic purposes occurring from glycolysis in the present studies that was not detected. It is of some interest that in the rat ileal preparation Dickens and

TABLE 4. *Oxidation of various substrates by ileum from various species of animals*

Added Substrate	Monkey		Hamster		Guinea Pig		Rat	
	μl O_2	$C^{14}O_2$	μl O_2	$C^{14}O_2$	μl O_2	$C^{14}O_2$	μl O_2	$C^{14}O_2$
None	52		133		155		60	
Glucose-C^{14}	69	1,808	164	4,300	139	2,377	77	1,827
Glutamine-C^{14}	72	5,038	154	5,200	149	10,289	66	2,371
Palmitate-C^{14}	61	31,470	143	110,000	180	22,855	45	10,186
Acetate-C^{14}	58	9,806	142	20,200	156	30,100	71	4,434

All values are μl/100 mg per hr or counts/min in CO_2/100 mg per hr. For details see Table 1.

Weil-Malherbe found an RQ of 0.78 in the presence of added glucose (1). In our studies ileum from the normal rabbit has consistently had an RQ of unity or greater; only rabbit ileum that has been damaged by cholera vibrio has an RQ of 0.89 (unpublished data).

The low RQ found by Dickens and Weil-Malherbe for rat ileum suggested that fatty acid oxidation might be a component of the respiratory metabolism of this tissue. Rao and Hansen reported, however, that stearate-C^{14} was not converted to $C^{14}O_2$ despite the fact that it was incorporated into tissue lipids of ileum in vitro (8). In the present studies various preparations of labeled palmitate have been used and there has been consistent oxidation to $C^{14}O_2$. Also this same palmitate is incorporated into tissue lipids from which the radioactive component can be hydrolyzed by alkali and after acidification extracted by hexane (unpublished data). The radioactivity in the hexane can be re-extracted with alkaline ethanol (6). These facts lead us to conclude that we are probably observing true oxidation of the added palmitate to $C^{14}O_2$ in the system that we used in the present studies. One experiment was performed that was identical to that shown in Table 3B except that stearic acid-1-C^{14} was the labeled substrate. Similar results were obtained although the radioactivity in the CO_2 was only one-half of that found for palmitate. Although palmitate was added at tracer concentration, acetate at substrate concentrations was vigorously oxidized by ileal preparations of all species studied. Although free acetate probably is not a physiological substrate in these animals in vivo, it seems reasonable to conclude that ileum from some animals can oxidize fatty acids.

In their report on the metabolism of the rat intestinal mucous membrane, Dickens and Weil-Malherbe noticed

a seasonal variation with respect to glycolysis (1). The present studies were begun in winter and during the moderately cold period the endogenous respiration was in the range of 40–50 μl 100 mg wet wt per hr. As the studies were continued through the spring and early summer (95 F), the endogenous respiration rose to a range of approximately 100 μl 100 mg wet wt per hr. Although added glucose and glutamine still stimulated respiration, the proportional response was much less. No change in technique or diet could be determined and therefore this may represent a seasonal variation.

The present observation that ileum in vitro has an appreciable endogenous respiration is not new. Dickens and Weil-Malherbe (1) measured appreciable oxygen consumption by rat ileum without added substrate. Newey et al. (7) made similar observations as well as demonstrating that rat ileum in vitro can convert labeled glucose to $C^{14}O_2$.

Additional experiments in this laboratory (unpublished data) have shown that after a 72-hr fast rabbit ileum still has a marked endogenous respiration (70 μl 100 mg per hr). The response to glucose and glutamine is the same as found with tissue from the fed animal. This would appear to indicate that the rabbit ileum is not easily depleted of endogenous stores of substrate for the support respiration b ̄ hr of starvation.

The findings of species differ   ᵇⁱˢ communication are compatible with earlie   :ch as the differences in succinoxidase activity   and guinea pig intestine (2) and the varying s   ep ability of intestine of various mammals to damage by aminopterin (9).

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